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LETTER



# Reaction of Cysteine(s) with Phenyldichloroarsine

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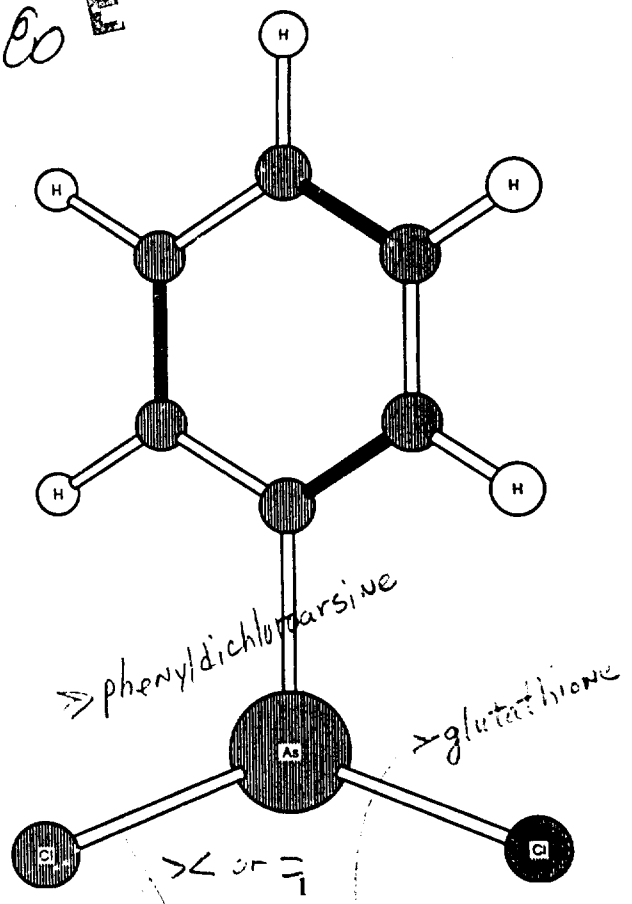


Organic arsenicals readily and preferentially react with sulfhydryl-containing compounds [1, 2]. Trivalent organic arsenicals are particularly potent cytotoxic compounds, presumably because they inhibit enzymic function by binding to sulfhydryl groups at the active sites of enzymes.

In our recent studies we showed that the trivalent organic arsenical phenyldichloroarsine (PDA; 1) will enter erythrocytes and bind to intracellular glutathione (GSH) via the sulfur atom of the central cystyl residue; the adduct formed is $\phi$-As(GS)$_2$ [3–5]. In the rate erythrocyte, a secondary PDA binding site was found in hemoglobin which contains additional sulfhydryl groups relative to human hemoglobin. The two sulfhydryl groups that bind PDA are thought to be the Cys-13 residues of the alpha chains [5]. This is puzzling because the two Cys-13 residues are spatially not in close proximity and 1:1 As:S adducts are not characteristically stable [2, 6]. When PDA reacts with GSH the 1:2 adduct ($\phi$-As(GS)$_2$) forms preferentially and the 1:1 adduct is detectable only when PDA is in large excess [3, 7]. We, therefore, postulated that in rat hemoglobin the second ligand of PDA could be a nitrogen (or oxygen) atom of a neighboring amino acid residue (for instance, Glu-116) [5].

In order to further our studies dealing with the interaction of organic arsenicals with biological sulfhydryl-containing molecules, we investigated the reaction of PDA with L-cysteine, and two derivatives of L-cysteine in methanol-$d_4$. We found that cysteine (in contrast to GSH in $H_2O$) formed a mono-cysteine adduct when the L-Cys/PDA ratio was $\leq$1:1 (2). When cysteine was present in two molar equivalents, the expected 2:1 adduct was formed via chelation of the two sulfur atoms of cysteine to the arsenic atom (3). Both L-cysteine methyl ester and *N*-acetyl-L-cysteine reacted like L-cysteine and formed a 1:1 adduct when the ratio was $\leq$1:1.

Keywords: Organic arsenicals, Cysteine, Phenyldichloroarsine, Biological, Sulfhydryl-containing molecules, Reprints

## Experimental

Phenyldichloroarsine was purchased from Research Inorganic/Organic Chemicals (Sun Valley, CA) and further purified by vacuum distillation [3]. L-Cysteine was obtained from Baker Chemical Company (Phillipsburg, NJ) and the two cysteine derivatives were obtained from Sigma Chemical Company (St. Louis, MO). Methanol-$d_4$ (99.90%) was a product of Merck, Sharpe & Dohme (West Point, PA).

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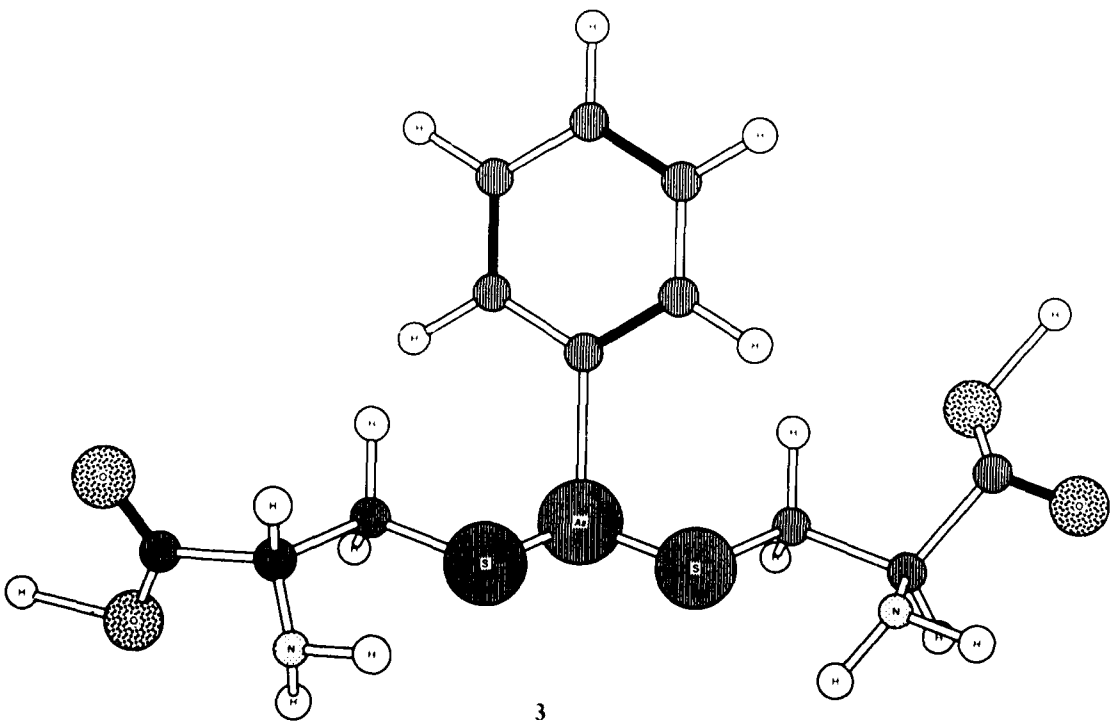


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For NMR studies, samples were prepared by the addition of PDA (using a positive displacement pipette) to methanol-$d_4$ in a 10-mm NMR tube. To these, measured quantifies of solid L-cysteine or the derivatives were added. The sample was then saturated with nitrogen, capped and shaken vigorously to ensure dissolution of the sample. $^{13}C$ NMR spectra were recorded on a Varian XL-300 spectrometer operating at 75.4 MHz, as previously described [3]. Further spectral conditions are given in the caption to Fig. 1.

## Results and Discussion

Figure 1 shows the effects of added L-cysteine on the proton-decoupled, natural abundance $^{13}C$ NMR spectrum of PDA in methanol-$d_4$. The upper trace shows the $^{13}C$ NMR spectrum of PDA. There

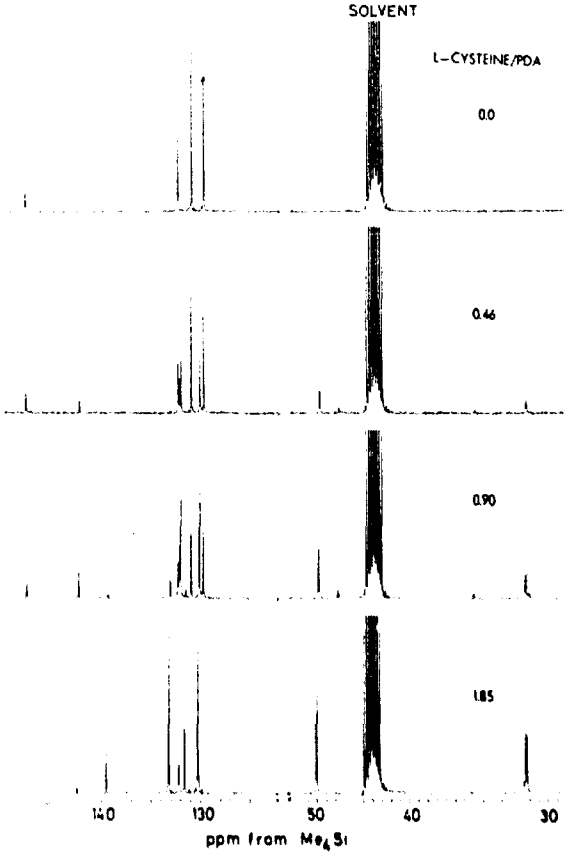


Fig. 1. The effects of added L-cysteine on the proton-decoupled, natural abundance $^{13}C$ NMR spectrum of PDA. The concentration of PDA was 125 mM in methanol-$d_4$. Solid L-cysteine was added to give the molar ratios of L-Cys/PDA as indicated in the right-hand portion of each trace. A new sample was prepared for each L-Cys/PDA ratio given.

are three resonances at ~132 ppm (representing five carbon atoms) and one less-intense resonance at about 148 ppm, representing the non-protonated aromatic carbon atom. Addition of L-cysteine (L-Cys) results in new resonances in the aliphatic region (Cys $C^{\beta}$ at ~32.5 ppm and Cys $C^{\alpha}$ at ~50 ppm) and carbonyl region (169 ppm; not shown in this Figure) of the spectrum. An indication of the complexity of the reaction(s) is seen in the aromatic region of the spectrum of PDA, especially the non-protonated carbon atom. As L-Cys is added, and the ratio of L-Cys/PDA approaches 1:1, three resonances are observed in the non-protonated carbon atom region of the spectrum; the dominant resonance occurs at 143 ppm. At an L-Cys/PDA ratio of ~2:1 the predominance of a single species is observed in the aromatic region of the spectrum; the dominant non-protonated aromatic carbon resonance now occurs at 139 ppm. The aliphatic carbon region shows that all of the resonances exist as doublets, indicating that a diastereotopic chemical shift difference has developed for this adduct because of a chiral center about the arsenic atom.

Almost identical results were obtained when L-cysteine methyl ester and *N*-acetyl-L-cysteine were used in our studies. For the *N*-acetyl-L-cysteine, the sample decomposed (loss of the acetyl moiety) over an extended period of time in the presence of PDA.

It is not obvious why GSH (in $H_2O$) preferentially forms a 2:1 adduct, whereas free cysteine clearly reacts in a 1:1 stoichiometry and proceeds to the 2:1 adduct when the L-Cys/PDA ratio is greater than 1:1. Assuming that the sulfur is responsible for the cysteine–arsenic bond, the structure of the 1:1 cysteine adduct is given in Fig. 1. We considered the possibility that the cysteine-PDA adduct might be stabilized if a second atom (e.g. oxygen or nitrogen) also served as a ligand with the arsenic and thus formed a ring structure. This does not appear to be the case, because both the methyl ester and the *N*-acetyl derivatives of L-cysteine also formed 1:1 adducts. Another possibility is that solvent plays a role in the adducts formed. Indeed, when we studied the reaction of PDA with GSH in methanol-$d_4$, we found similar results to those observed for the reaction of PDA with L-cysteine and the derivatives of L-cysteine. Although a solvent effect may explain the above reactions, the result obtained with rat hemoglobin is still anomalous.

## Acknowledgement

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